

**METHOD FOR IMMOBILISING AND/OR CRYSTALLISING BIOLOGICAL
MACROMOLECULES ON CARBON NANOTUBES AND USES**

The present invention relates to a method of
5 attaching and/or crystallizing macromolecules, to the
chemical reagents used in the said method, to the
products obtained as well as to the applications of the
said products in the field of materials and of
structural biology, in particular as biosensors or as
10 biomaterials.

The knowledge of the structure of proteins and
in particular of their active sites is essential for
understanding their mechanism of action. Several
methods are available for carrying out such studies: X-
15 rays, NMR, electrocrystallography (2D crystallization).

For carrying out the crystallization proper,
the technique of two-dimensional crystallization on a
lipid film or monolayer, at the air/water interface
(E.E. Ugziris et al., Nature, 1983, 301, 125-129),
20 allows the formation of self-organized systems of
biological macromolecules (crystals) and the
determination of the structures of these molecules by
electron microscopy analysis of the crystals obtained.

This method consists in creating a lipid
25 monolayer at the level of an air/liquid interface, the
lipids being selected so as to interact with the
proteins, present in the liquid phase, which attach to
the lipids and then form an organized network.

The attachment of the proteins to the lipids of
30 the monolayer involves chemical interactions at the
level of the polar head of the lipids. These
interactions are either aspecific, the lipids
possessing charged polar ends, giving rise to
crystallization through ionic interactions, or
35 specific. In the latter case, the polar head of the
lipids carries ligands exhibiting high affinity with
the proteins to be attached.

In particular, it has been possible to show that soluble proteins can two-dimensionally crystallize on lipid films which are charged, or which are functionalized by a ligand for the protein studied
5 (B.J. Jap et al., Ultramicroscopy, 1992, 46, 45-84).

More recently, lipids functionalized by metal complexes such as nickel complexes (E.W. Kubalek et al., J. Struct. Biol., 1994, 113, 117-123) have made it possible to crystallize so-called histidine tagged
10 fusion proteins. These proteins indeed possess, at their N- or C-terminal end, a sequence composed of several histidines. It has been possible to show that the attachment of such proteins to a lipid-nickel was due to a strong interaction between the nickel complex
15 and the poly-histidine sequence (C. Vénien-Brian et al., J. Mol. Biol., 1997, 274, 687-692). Such functionalized lipids have made it possible to obtain crystallization, in particular in the case where an appropriate ligand was not available.

20 However, the crystallization of proteins on lipid films has the disadvantage of being relatively random and of depending on many factors, which are difficult to control simultaneously:

- the ligand carried by the lipids should be
25 sufficiently accessible in order to be able to interact with the proteins. This accessibility depends on the length of the spacer arm between the lipid and the ligand: too short, it gives rise to a penetration of the protein inside the lipid layer; too long, it
30 confers an extremely high degree of freedom on the bound protein and increases the incidence of defects in the crystal;

- the lipid monolayer should be sufficiently fluid in order to confer a sufficient lateral and
35 rotational mobility on the bound protein, thus allowing the proteins to organize relative to each other and to develop intermolecular contacts, so as to give rise to the crystal;

- another difficulty, inherent to crystallization on a lipid monolayer, relates to the stability of the monolayer; indeed, the stability of the air/liquid interface is difficult to control. In addition, the lipid monolayer should remain stable, not only before the attachment of the proteins, but also after their attachment, in order to allow the spatial organization of the proteins;

- for the microscopy study which follows the crystallization step, it is necessary to produce a multitude of planes, because of the planar nature of the structure obtained.

Consequently, the inventors set themselves the aim of providing a method which makes it possible to attach in solution and to optionally induce self-organization of macromolecules which is more suitable for the requirements of practical use than the 2D crystallization methods previously used.

The subject of the present invention is a method for the attachment and/or self-organization of biological macromolecules, characterized in that it essentially comprises the incubation, without stirring, for at least 15 minutes, of a macromolecule in solution with nanotubes of carbon closed at their ends, under suitable temperature and pH conditions.

Nanotubes were discovered in 1991 (S. Ijima, *Nature* 1991, **354**, 54-56); since then, they have generated a lot of interest, in particular because of their mechanical properties: high mechanical resistance (M.M.J. Treacy et al., *Nature* 1996, **381**, pp. 678-680) and electronic properties: conductor or semiconductor property (J.W.G. Wildöer et al., *Nature* 1998, **391**, 59-62; T.W. Odom et al., *Nature*, 1998, **391**, 62-64).

Several methods of preparing nanotubes have been described, including that by T.W. Ebbesen et al. (*Nature*, 1992, **358**, 220-222), which makes it possible to obtain a high yield. Methods of purifying nanotubes have also been described (H. Hiura et al., *Adv. Mater.*, 1995, **7**, 275-276; J-M Bonard et al., *Adv. Mater.*, 1997,

9, 827-831 and G.S. Duesberg et al., *Chem. Commun.* 1998, 435-436); these various methods make it possible to obtain the desired quantities of nanotubes. Methods for the chemical functionalization of nanotubes of carbon have also been described (International Application PCT WO97/32571).

Other methods for the chemical functionalization of nanotubes have also been described; there may be mentioned for example TSANG S.C. et al., *Journal of the Chemical Society, Chemical Communications*, 1995, 17, 1803-1804 and DAVIS J.J. et al., *Inorganica Chimica Acta*, 1998, 272, 1, 2, 262-266.

However, they involve chemical reactions which either dramatically modify the geometry of the nanotubes (opening of the ends, partial destruction of the outer sheets), or destroy the intrinsic physical properties of the nanotubes and consequently do not allow organization of biological macromolecules such as proteins, on the nanotubes. Nanotubes modified by such destructive methods are not therefore suitable for adsorption and/or self-organization at their outer surface of synthetic products or of biological macromolecules.

Depending on the technique and the conditions used, several structures of nanotubes may be prepared: the nanotubes have in particular so-called multi-wall nanotube structures (MWNT) or single-wall nanotube structures (SWNT) of graphite. They can be completely, partially or not at all oxidized.

Thus, the nanotubes are, from a chemical point of view, polymers composed solely of carbon and which may comprise up to a million atoms. In accordance with the laws of the chemistry of carbon, the atoms of a nanotube are linked via a solid covalent bond and each atom possesses exactly three neighbours. Thus, regardless of its length, a nanotube is obliged to close at its ends, so as not to leave any chemical bond alone there. In general, its diameter is generally

between 1 and 30 nm and its length may be up to several micrometres.

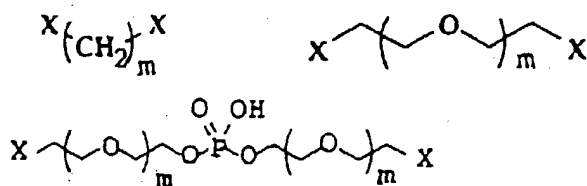
From a physical point of view, nanotubes can be defined as carbon crystals extending in a single direction, the repeating unit having the symmetry of a helix (B.I. Yakobson et al., American Scientist, 1997, 85, 324-337).

According to an advantageous embodiment of the said method, the said biological macromolecules are in particular soluble, membrane or transmembrane proteins, enzymes, antibodies, antibody fragments or nucleic acids.

According to another advantageous embodiment of the said method, the said nanotubes of carbon are functionalized by physical adsorption of a chemical reagent of general formula **H-E-L**, in which:

- **H** represents a hydrophilic group, selected from the positively or negatively charged groups; ligands or analogues of biological macromolecules, such as, without limitation, biotin, novobiocin, retinoic acid, steroids, antigens; organometallic complexes interacting with amino acids or nucleic acids, such as complexes of copper, zinc, nickel, cobalt, chromium, platinum, palladium, iron, ruthenium or osmium with ligands such as IDA, NTA, EDTA, bipyridine or terpyridine, the said ligands being optionally functionalized with alkyl groups for bonding to E (at the level of X); positively or negatively charged groups are understood to mean, without limitation: ammoniums, carboxides, phosphates, sulphonates; the following groups may be mentioned for example: $-N(CH_3)_3^+$ or $-CO_2^-$.

- **E** represents a spacer arm, selected from C_1 - C_{10} carbon chains, optionally substituted with alkyl groups or otherwise, having unsaturations or polyoxyethylene units which may have or otherwise in the middle of the chain phosphate groups, such as:

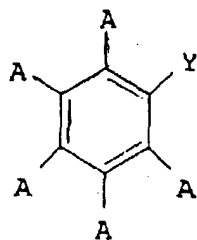


in which:

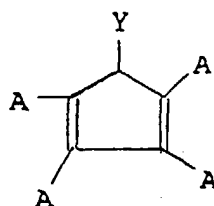
m represents an integer from 1 to 10,

5 X represents O, NHCO, OCO, COO, CONH, S, CH₂ or NH and constitutes, at the ends of the said carbon chains, organic functions for adhesion of the ester, amide, ether or thioether type;

- L represents a lipid unit with one or more
10 chains of variable length, in the form of C₁₂-C₂₀ having unsaturations or otherwise; an aromatic group of formula Ar₁ or of formula Ar₂:



Ar₁



Ar₂

15

in which:

A represents a hydrogen atom, one of the following groups: alkyl, CF₃, NO₂, NH₂, OH, O-alkyl, S-alkyl, COOH, halogen, an aromatic ring or an aromatic
20 heterocycle in the form of C₄-C₆, optionally polysubstituted with electron-donating groups of the alkyl type or electron-attracting groups of the CF₃ or halide type; L represents for example one of the following aromatic groups: benzyl, naphthyl,
25 anthracenyl, fluoroenyl, tetrabenzofluoroenyl, and

Y represents a bond with E.

For the purposes of the present invention, alkyl is understood to mean linear or branched or optionally substituted C₁-C₆ alkyl groups.

Surprisingly, both untreated (non-functionalized) nanotubes of carbon and nanotubes of carbon functionalized by non-destructive methods, as defined above, can be used in the method according to the invention.

The functionalization according to the present invention is, surprisingly, non-destructive for the nanotubes; in particular, it avoids the opening of their ends.

It is possible with such a method to adsorb and/or to self-organize at the outer surface of the nanotubes of carbon either synthetic products or biological macromolecules.

Indeed, the present invention makes it possible to induce the formation of arrangements of macromolecules (self-organization) such as proteins, with a helical symmetry.

According to another embodiment of the said method, the said solution consists of a solvent for solubilizing the said biological macromolecules, which is aqueous or aqueous-alcoholic and which optionally contains at least one detergent, depending on the biological macromolecule to be crystallized.

According to another advantageous embodiment of the said method, the incubation conditions are preferably the following: incubation at room temperature, for 15 minutes to 48 hours, at a pH of between 5.5 and 8.5.

Surprisingly, the said method makes it possible to obtain arrangements of biological macromolecules which allow structural studies by electronic microscopy and the preparation of novel nano materials which can be used for their physical, electrical or biological properties.

Such a method has the advantage of making the crystallization of proteins reproducible; in particular, it is easy, in the case where a protein does not crystallize in the presence of nanotubes of a given diameter, to use the method with nanotubes of

different diameter; indeed, the crystallization of a given protein depends on the diameter of the nanotubes.

However, in the present invention, it is possible to vary the diameter of the nanotubes and to
5 use equally well multi-wall or single-wall nanotubes of carbon which are completely, partially or not at all oxidized.

Also surprisingly, in the method according to the invention, the attachment or the crystallization of
10 macromolecules on the nanotubes of carbon may be, under the appropriate experimental conditions, as defined above, either spontaneous, that is to say in the absence of any other synthetic products, or induced by addition of a chemical reagent H-E-L, as defined above.

Also surprisingly, the various factors which
15 may come into play in order to allow a reproducible crystallization are, as already specified above, the following: the concentration of the samples, the choice of the solvents, the ionic restraint, the pH of the solutions, the incubation time and the diameter of the
20 nanotubes.

Both the reagents in which **L** represents a lipid unit with one or more chains of variable length in the form of C_{12} - C_{20} , having unsaturations or otherwise, and
25 the reagents in which **L** represents an aromatic group of formula Ar_1 or of formula Ar_2 , make it possible to obtain functionalized nanotubes suitable for the arrangement of macromolecules at their surface; however, the reagents in which **L** represents an aromatic
30 group of formula Ar_1 or of formula Ar_2 are particularly preferred.

The subject of the present invention is also bionanomaterials, characterized in that they essentially consist of nanotubes of carbon, on which
35 biological macromolecules are attached by means of non-covalent bonds.

The subject of the present invention is also bionanomaterials, characterized in that they essentially consist of nanotubes of carbon, on which

biological macromolecules are self-organized in a crystalline form.

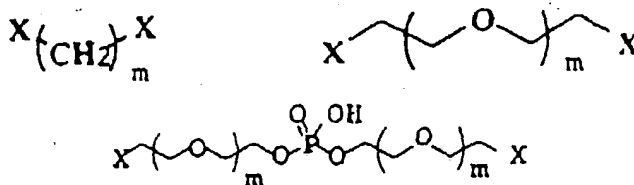
According to an advantageous embodiment of the said bionanomaterials, they are obtained with the aid of a method as defined above.

The subject of the present invention is in addition the applications of the said bionanomaterials to the structural study of the biological macromolecules which are associated with them, as biological reagent and more particularly as immunological reagent and as biosensors or bioconductors.

The subject of the present invention is in addition a chemical reagent capable of being physically adsorbed on nanotubes of carbon, characterized in that it has the general formula **H-E-L**, in which:

- **H** represents a hydrophilic group selected from the positively or negatively charged groups; ligands or analogues of biological macromolecules; organometallic complexes interacting with amino acids or nucleic acids and whose ligands are optionally functionalized with alkyl groups for bonding to **E**;

- **E** represents a spacer arm, selected from C_1 - C_{10} carbon chains, optionally substituted with alkyl groups, having unsaturations or otherwise or polyoxyethylene units which may have or otherwise in the middle of the chain phosphate groups, such as:



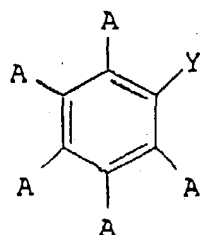
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in which:

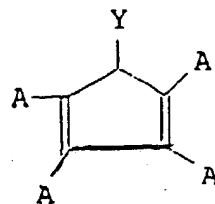
m represents an integer from 1 to 10,

X represents O, NHCO, OCO, COO, CONH, S, CH_2 or NH and constitutes, at the ends of the said carbon chains, organic functions for adhesion of the ester, amide, ether or thioether type;

- L represents an aromatic group of formula Ar_1 or of formula Ar_2 :



Ar_1



Ar_2

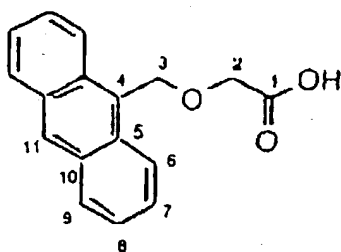
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in which:

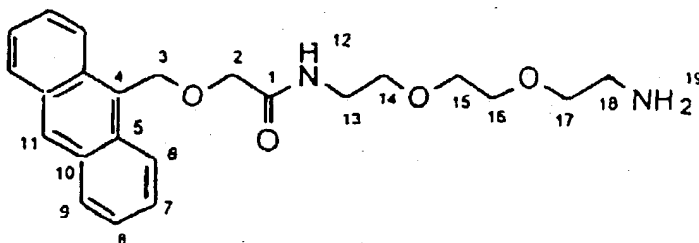
A represents a hydrogen atom, one of the following groups: alkyl, CF_3 , NO_2 , NH_2 , OH , O-alkyl, S-alkyl, $COOH$, halogen, an aromatic ring or an aromatic heterocycle in the form of C_4-C_6 , the said rings being optionally polysubstituted with electron-donating groups of the alkyl type or electron-attracting groups of the CF_3 or halide type; and

Y represents a bond with E.

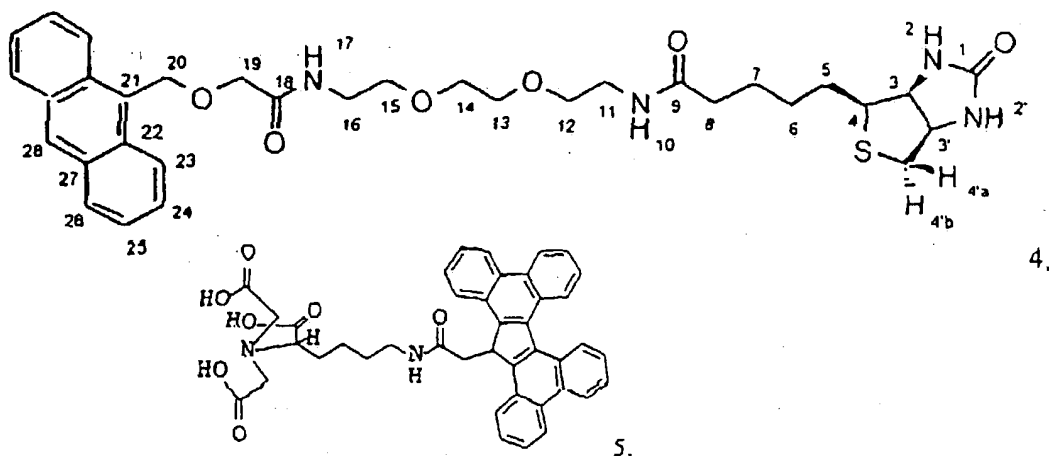
15 According to an advantageous embodiment of the said chemical reagent, it has one of the following structures:



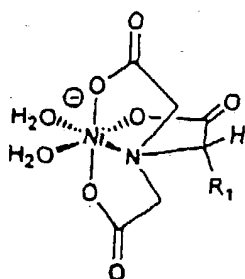
1,



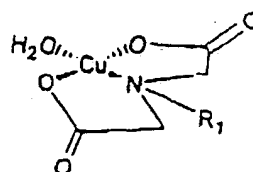
3,



According to another advantageous embodiment of the said chemical reagent, H is selected from the following organometallic complexes:



Ni-NTA complex



Cu-IDA complex

10

with R_1 = organic group for bonding to E.

In addition to the preceding arrangements, the invention further comprises other arrangements, which will emerge from the description which follows, which refer to exemplary embodiments of the method which is the subject of the present invention as well as to the accompanying drawings, in which:

- Figure 1 illustrates the crystallization of a biological macromolecule on a nanotube of carbon, by addition (physical adsorption) of a chemical reagent;

- Figure 2 illustrates a structure of a chemical reagent used to functionalize, by physical adsorption, the nanotubes of carbon;

- Figure 3 represents nanotubes with a diameter of close to 10 nm, covered with helical crystals of streptavidin;

- Figure 4 represents nanotubes covered with
5 helical crystals of HupR protein.

It should be clearly understood, however, that these examples are given solely by way of illustration of the subject of the invention and do not constitute in any manner a limitation thereto.

10 **Example 1: Self-organization on multi-wall nanotubes of carbon of molecules of streptavidin in the form of helical crystals**

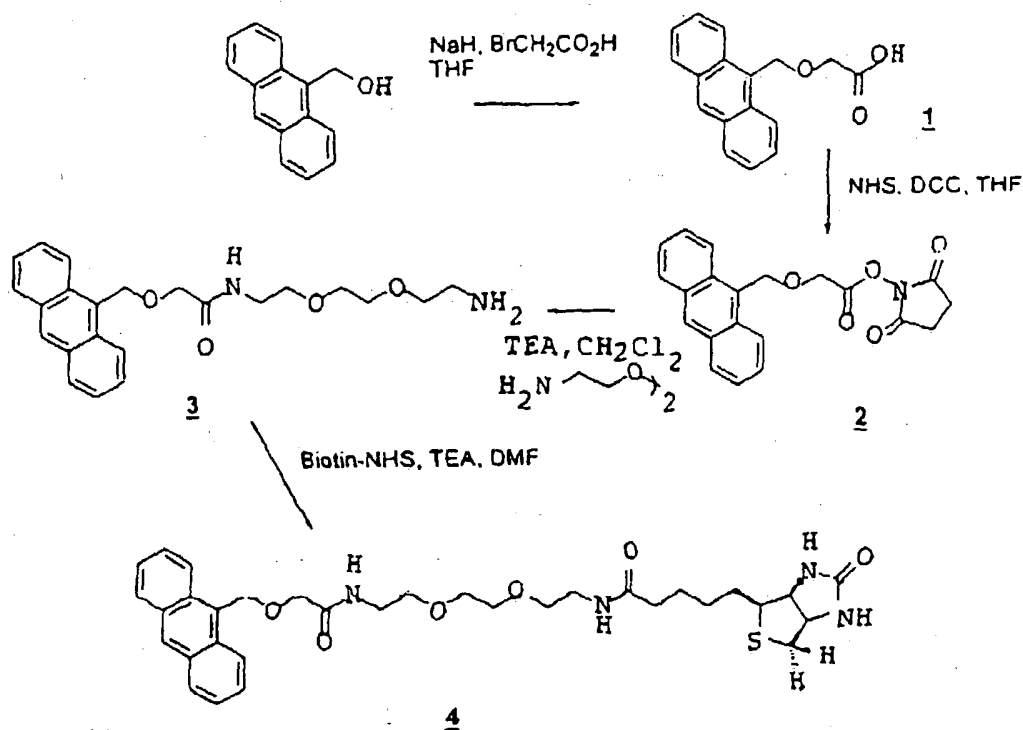
The multi-wall nanotubes of carbon (MWNT) used are produced by decomposition of a graphite electrode
15 with an electric arc (T.W. Ebbesen et al., *Nature* 1992, Vol. 358, pp. 220-222). After sonication of a solution (2 mg/ml) of nanotubes of carbon, 20 µg of MWNT are collected and dried with an ethane gas stream so as to be finally resuspended in 20 µl of a water/methanol
20 mixture (40% of methanol by volume). After sonication, 20 µl of an aqueous solution of streptavidin (10 µg/ml) are added and the mixture is left, without stirring or vortexing, at room temperature for 45 minutes. 5 µl of the suspension of nanotubes of carbon are then
25 deposited on an electron microscopy grid coated with a carbon film. After negative staining of the sample with a solution of uranyl acetate, the grid is observed in an electron microscope (Philips CM120). It was possible to confirm that the nanotubes with a diameter of close
30 to 10 nm are coated with helical crystals of streptavidin.

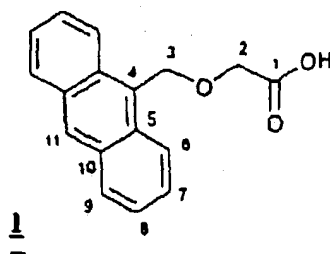
Example 2: Self-organization on multi-wall nanotubes of carbon of molecules of "histidine-tagged" HupR in the form of helical crystals

35 The multi-wall nanotubes of carbon (MWNT) used are produced by decomposition of a graphite electrode with an electric arc (T.W. Ebbesen et al., *Nature* 1992, Vol. 358, pp. 220-222). After sonication of a solution (2 mg/ml) of nanotubes of carbon, 20 µg of MWNT are

collected and dried with an ethane gas stream so as to be finally resuspended in 20 μ l of aqueous buffer (10 mM Tris; pH = 7.5; 350 mM NaCl). After sonication, 20 μ l of an aqueous solution of histidine-tagged HupR protein (10 μ g/ml) from *Rhodobacter Capsulatus* are added and the mixture is left, without stirring or vortexing, at room temperature for 25 minutes. 5 μ l of the suspension of nanotubes of carbon are then deposited on an electron microscopy grid coated with a carbon film. After negative staining of the sample with a solution of uranyl acetate, the grid is observed in an electron microscope (Philips CM120). It was possible to note that a large number of nanotubes are coated with helical crystals of HupR protein.

Example 3: Preparation of a biotinylated chemical reagent





PROCEDURE

2.1 g (10 mmol, 1 eq) of 9-anthracenemethanol
5 in 20 ml of THF are added at 0°C to a suspension of
1.2 g (30 mmol, 3 eq) of sodium hydride at 60% in oil
in 20 ml of THF. The mixture is stirred for one hour
under reflux and then the temperature is reduced to 0°C
in order to add 1.4 g (10 mmol, 1 eq) of bromoacetic
10 acid in solution in 20 ml of THF. This solution is
further stirred for 5 minutes at 0°C and then for one
hour at room temperature before being heated under
reflux for 16 hours. After cooling, the reaction is
stopped by addition of 40 ml of a saturated aqueous
15 ammonium chloride solution followed by 10 ml of an
aqueous hydrochloric acid solution. The reaction medium
is then extracted with twice 50 ml of ether. After
drying over sodium sulphate, the organic phase is
evaporated to dryness in order to give after
20 chromatography on silica (hexane/EtOAc/ AcOH: 70/30/1)
1.212 g of a yellow solid (yield: 45.5%).

EF: C₁₇H₁₄O₃

m.p.: 266.299 g/mol

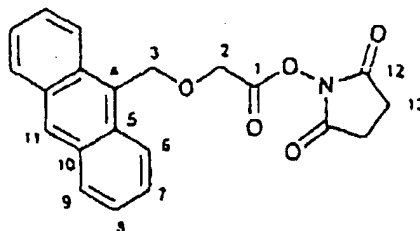
TLC: R_f (EtOAc/Hex/AcOH; 80/20/1): 0.56;

¹H NMR (300.13 MHz, acetone d₆): δ 11.2 (bs, 1H, H₁);
25 8.64 and 8.10 (d and d, 4H, J = 8.8 Hz, J = 7.9 Hz, H₆,
H₉); 8.61 (s, 1H, H₁₁); 7.5-7.7 (m, 4H, H₇, H₈); 5.68 (s,
2H, H₃); 4.41 (s, 2H, H₂);

¹³C NMR (75.47 MHz, acetone d₆): δ 176.27 (1C, C₁);
136.51, 136.16 and 133.29 (5C, C₄, C₅, C₁₀); 133.75,
30 131.10, 130.14 and 129.73 (9C, C₆, C₇, C₈, C₉, C₁₁);
71.88 (1C, C₃); 69.81 (1C, C₂):

MS (70eV/DCI/intensity %): m/e: 191 (100,
[M-OCH₂CO₂H]⁺); 266 (12, [M+1]⁺); 284 (58, [M+18]⁺);

2,5-Dioxopyrrolidin-1-yl (anthracen-9-ylmethoxy) acetate 2:



5

2

PROCEDURE

510 mg (2.47 mmol, 1 eq) of DCC in solution in 20 ml of THF are added at 0°C to a solution of 650 mg (2.44 mmol, 1 eq) of (anthracen-9-ylmethoxy)acetic acid 1 and 300 mg of NHS (2.61 mmol, 1.07 eq) in 30 ml of THF. The mixture is stirred overnight at room temperature. The reaction medium is then filtered and then evaporated. The residue obtained is taken up in 50 ml of absolute ethanol in order to give after filtration 727 mg of 2,5-dioxopyrrolidin-1-yle (anthracen-9-ylmethoxy)acetate 2 in the form of a white solid (yield: 82%).

EF: C₂₁H₁₇NO₅

m.p.: 363.374 g/mol

20 TLC: R_f (EtOAc/Hex; 50/50): 0.33;

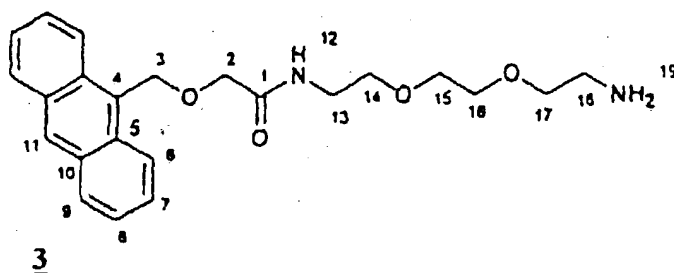
¹H NMR (300.13 MHz, CDCl₃): δ 8.51 (s, 1H, H₁₁); 8.42 and 8.02 (d and d, 4H, J = 9.0 Hz, J = 8.4 Hz, H₆, H₉); 7.59 and 7.48 (dd and dd, 4H, H₇, H₈); 5.71 (s, 2H, H₃); 4.53 (s, 2H, H₂); 2.90 (s, 4H, H₁₃);

25 ¹³C NMR (75.47 MHz, CDCl₃): δ 168.49 (2C, C₁₂); 166.16 (1C, C₁); 131.13, 128.94 and 126.36 (5C, C₄, C₅, C₁₀); 128.82, 126.51, 124.88 and 123.83 (9C, C₆, C₇, C₈, C₉, C₁₁); 65.28 (1C, C₃); 64.57 (1C, C₂); 25.39 (2C, C₁₃);

MS (70eV/DCI/intensity %): m/e: 381 (100, [M+18]⁺);

30

N-{2-[2-(2-Aminoethoxy)ethoxy]ethyl}-2-(anthracen-9-ylmethoxy)acetamide 3:



PROCEDURE

1.75 ml of TEA and 91 mg (0.25 mmol, 1 eq) of 2,5-dioxopyrrolidin-1-yl (anthracen-9-ylmethoxy) acetate 2 in solution in 5 ml of CH₂Cl₂ are added to a solution of 370 mg (2.50 mmol, 10 eq) of 2,2'-(ethylenedioxy)diethylamine in 20 ml of CH₂Cl₂. The mixture is stirred for 7 hours at room temperature. The reaction medium is then evaporated. The residue obtained is washed with 50 ml of water and then 50 ml of an aqueous potassium hydroxide solution (0.1 N). The organic phase is dried, evaporated and concentrated under vacuum to give after chromatography on silica (CH₂Cl₂/MeOH/TEA; 90/10/1), 55 mg of N-{2-[2-(2-aminoethoxy)ethoxy]ethyl}-2-(anthracen-9-ylmethoxy)acetamide 3 in the form of a yellow oil (yield: 55%).

EF: C₂₃H₂₈N₂O₄

m.p.: 396.491 g/mol

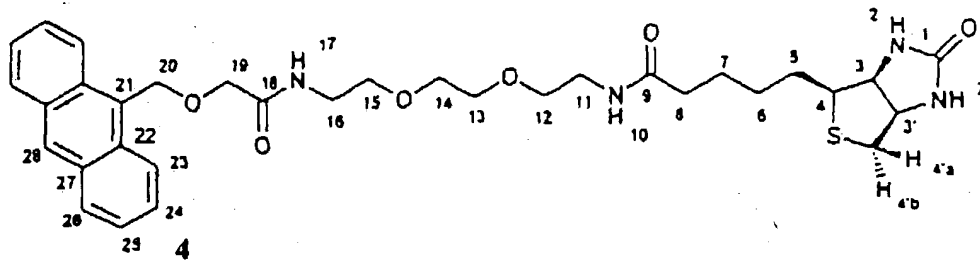
20 TLC: R_f (CH₂Cl₂/MeOH/TEA; 90/10/1): 0.28;

¹H NMR (300.13 MHz, CDCl₃): δ 8.44 (s, 1H, H₁₁); 8.29 and 7.98 (d and d, 4H, J = 9.0 Hz, J = 8.4 Hz, H₆, H₉); 7.53 and 7.45 (dd and dd, 4H, H₇, H₈); 6.93 (t, 1H, J₆₋₉ = 5.4 Hz, 1H, H₁₂); 5.51 (s, 2H, H₃); 4.13 (s, 2H, H₂); 3.3-3.5 (m, 8H, H₁₄, H₁₅, H₁₆, H₁₇); 3.25 (t, J = 5.1 Hz, 2H, H₁₃); 2.7-2.8 (m, 2H, H₁₉); 2.64 (t, 2H, J = 5, 1 Hz, H₁₈);

¹³C NMR (75.47 MHz, CDCl₃): δ 169.49 (1C, C₁); 131.11, 130.73 and 127.08 (5C, C₄, C₅, C₁₀); 128.92, 128.69, 126.39, 124.87 and 123.64 (9C, C₆, C₇, C₈, C₉, C₁₁); 72.10, 69.89, 69.78, 69.47 and 69.33 (5C, C₃, C₁₄, C₁₅, C₁₆, C₁₇); 65.15 (1C, C₂); 41.03 (1C, C₁₃); 38.31 (1C, C₁₈);

MS (70eV/DCI/intensity %): m/e: 397 (100, [M+1]⁺);

N-(2-[2-(2-(Aminobiotin)ethoxy)ethoxy]ethyl)-2-(anthracen-9-ylmethoxy)acetamide 4:



5

PROCEDURE

1 ml of TEA and 40 mg (0.12 mmol, 1.2 eq) of biotin-NHS in solution in 5 ml of DMF are added to a solution of 40 mg (0.1 mmol, 1 eq) of *N*-(2-[2-(2-aminoethoxy)ethoxy]ethyl)-2-(anthracen-9-ylmethoxy)acetamide 3 in 5 ml of DMF. The mixture is stirred for 16 hours at room temperature. The reaction medium is concentrated under vacuum in order to give after chromatography on silica (CH₂Cl₂/MeOH/TEA; 95/5/1), 55 mg of *N*-(2-[2-(2-(aminobiotin)ethoxy)ethoxy]ethyl)-2-(anthracen-9-ylmethoxy)acetamide 4 in the form of a yellow oil (yield: 89%).

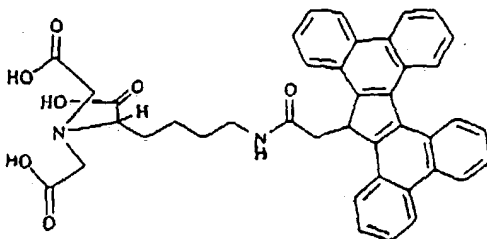
EF: C₃₃H₄₂N₄O₆S m.p.: 622.793 g/mol
 20 TLC: R_f (CH₂Cl₂/MeOH; 90/10): 0.5;
¹H NMR (300.13 MHz, CDCl₃): δ 8.48 (s, 1H, H₂₈); 8.31 and 7.01 (d and d, 4H, J = 9.0 Hz, J = 8.4 Hz, H₂₃, H₂₆); 7.55 and 7.47 (dd and dd, 4H, H₂₄, H₂₅); 6.89 (t, 1H, J₁₆₋₁₇ = 5.0 Hz, H₁₇); 6.90 (s, 1H, H₂); 6.53 (t, 1H, J₁₀₋₁₁ = 5.0 Hz, H₁₀); 5.67 (s, 1H, H_{2'}); 5.55 (s, 2H, H₂₀); 4.31 (m, 1H, H₃); 4.17 (s, 2H, H₁₉); 4.15 (m, 1H, H_{3'})^γ; 3.2-3.5 (m, 12H, H₁₁, H₁₂, H₁₃, H₁₄, H₁₅, H₁₆); 2.97 (dt, 2H, H₄); 2.75 (dt, 1H, J_{3'-4'a} = 4.8 Hz, J_{4'a-4'b} = 12.7 Hz, H_{4'a}); 2.2 (d, 1H, J_{4'a-4'b} = 12.7 Hz, H_{4'b}); 30 2.08 (t, 2H, J₇₋₈ = 7.4 Hz, H₈); 1.2-1.7 (m, 6H, H₅, H₆, H₇);
¹³C NMR (75.47 MHz, CDCl₃): δ 173.00 (1C, C₁); 169.56 (1C, C₁₈); 163.76 (1C, C₉); 131.14, 130.73 and 127.05 (5C, C₂₁, C₂₂, C₂₇); 128.98, 128.75, 126.45, 124.93 and

123.59 (9C, C₂₃, C₂₄, C₂₅, C₂₆, C₂₈); 69.74, 69.55, 69.23
and 65.25 (6C, C₁₂, C₁₃, C₁₄, C₁₅, C₁₉, C₂₀); 61.50 and
59.88 (2C, C₃, C_{3'}); 55.32 (1C, C₄); 40.18 (1C, C_{4'});
38.76, 38.33 and 35.65 (3C, C₈, C₁₁, C₁₆); 27.95 and
5 27.81 (2C, C₅, C₇); 25.30 (1C, C₆);
MS (70eV/DCI/intensity %): m/e: 623 (100, [M+1]⁺); 640
(8, [M+18]⁺);

Example 4: Physical adsorption on nanotubes of carbon
of a chemical reagent, called hereinafter CR174, having
10 the structure H-E-L

Protocol: 1 to 20 µl of a solution of chemical
reagent called CR174 and whose chemical formula is
illustrated below, (1 mg/ml) in methanol are added to
20 µl of a solution of nanotubes of carbon (10 mg/ml in
15 methanol), freshly sonicated. The mixture is then
stirred by sonication and then evaporated to dryness by
a stream of ethane gas. 40 µl of Tris buffer (20 mM,
pH 7.5; 50 mM NaCl) are added to the dry nanotubes of
carbon and the suspension is remixed by sonication. The
20 suspension may be optionally centrifuged and washed
several times with 500 µl of buffer in order to remove
the excess reagent not adsorbed on the nanotubes of
carbon.

The physical adsorption of the reagent CR174 on
25 the nanotubes of carbon was able to be demonstrated by
electron microscopy with positive staining. The
presence of molecules of reagents on the nanotubes of
carbon results in the appearance of black spots. These
black spots are absent in the absence of the chemical
30 reagent CR174 (or 5).



As is evident from the preceding text, the
invention is not at all limited to its embodiments,

implementations and applications which have just been described more explicitly; on the contrary, it encompasses all the variants which may occur to a specialist in this field, without departing from the
5 framework or the scope of the present invention.